binding of Adenosine mono-phosphate (AMP) molecules being label-free. We found an increase in rupture force of 11 pN in the presence of AMP molecules in both binding pockets. The route to use a split aptamer probes in AFS enables us to determine precisely the dissociation constant of the AMP-aptamer system (3.7 ± 1.5 μM) by increasing the AMP concentration in solution. The concept of a split aptamer binding single small target also worked for the cocaine and antibiotics molecules.

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Complete Characterization of the Mechanochemical Cycle of a Homomeric Ring ATPase

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Understanding how multimeric ring ATPases couple chemical transitions to mechanical motion and coordinate their subunits is essential for elucidating the functions of these important molecular machines. Here we used high-resolution optical tweezers to study one such ATPase - the bacteriophage phi29 DNA packaging motor. This pentameric motor is known to translocate DNA by cycling through a dwell phase, in which subunits load ATPs, and a burst phase, in which 10 base pairs of DNA are packaged in four 2.5-base-pair steps. By monitoring packaging in buffers containing various nucleotides and nucleotide analogs, we determined the exact timing of chemical transitions (nucleotide hydrolysis and product release) with respect to the mechanical phases of the motor's cycle. Our results reveal an intricate coordination mechanism with multiple levels of communication between neighboring subunits. ADP release and ATP binding occur in a concerted alternating fashion, with ATP binding to one subunit facilitating the release of ADP in the following subunit. ATP hydrolysis also appears to occur sequentially around the ring through an intersubunit allosteric effect. Moreover, our data suggest that all five subunits of the ring bind and hydrolyze ATP. Only four out of the five hydrolysis events are coupled to DNA translocation, while the fifth subunit consumes ATP to fulfill a regulatory role, resetting the motor for the next mechanochemical cycle. The phi29 motor is able to tolerate a single inactive subunit and still function, albeit at a much slower pace. These new findings allow us to present the most complete mechanochemical model of a homomeric ring ATPase to date, which should provide insight into the operating principles of other ring-shaped ATPases.

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Direct Observation of Helix Staggering, Sliding, and Coiled Coil Misfolding

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The biological functions of coiled coils generally depend on efficient folding and perfect pairing of their α-helices. Dynamic changes in the helical registry that lead to staggered helices have only been proposed for a few special systems and not found in generic coiled coils. Here, we report our observations of multiple staggered helical structures of two canonical coiled coils. The partially folded structures are formed predominantly by coiled coil misfolding and occasionally by helix sliding. Using high-resolution optical tweezers, we characterized their energies and transition kinetics at a single-molecule level. The staggered states occur less than 2% of the time and about 0.1% of the time at zero force. We conclude that dynamic changes in helical registry may be a general property of coiled coils. Our findings should have broad and novel implications in functions and dysfunctions of proteins containing coiled coils.

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Differential Mechanical Stability of Filamin a Rod Segments and Domain Pair Interaction

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Filamin A (FLNa), an actin cross-linking protein, consists of two subunits that dimerize through C-terminal self-association domain. Each subunit contains an N-terminal spectrin-related actin-binding domain followed by 24 immunoglobulin-like (Ig) repeats. Two flexible hinges separate the 24 Ig repeats into rod 1 (repeats 1-15), rod 2 (repeats 16-23), and self-association domain 24. Rod 1 is like a linear array of Ig repeats, whereas rod 2 is more compact due to inter-domain interactions. FLNa not only support the tension of actin network but also interact with many transmembrane and signaling proteins mostly in the rod 2 segment.

Prompted by recent reports suggesting that interaction of FLNa with its binding partners is regulated by mechanical force, we examined mechanical properties of FLNa domains by magnetic tweezers. The three segments of Ig 1-8, Ig 9-15, Ig 16-23 are unfolded at different forces under the same loading rate. Remarkably, we found that repeats 16-23 are susceptible to ~10 pN force, while the repeats in the rod 1 segment can withstand significantly higher forces. In rod 2, nearest neighboring domains 16-17, 18-19, and 20-21 form domain pairs. Cryptic binding sites in rod 2 can be blocked by inter-domain interactions. For example, A strand of domain 20 blocks the binding site of β-integrin tail on domain 21. If force can unpeel strand A of domain 20 from domain 21, the binding site of β-integrin tail will be exposed, and the binding will be facilitated by the force. This is just one kind of proposed mechanism of force sensor. The specific domain pair interaction between 20 and 21 was studied by magnetic tweezers. Preliminary data shows that the disruption of the domain pair interaction between domain 20 and 21 occurs at ~15 pN.

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Temperature, Length Scale and Surface Dependence of Single Polymer Hydrophobic Hydration

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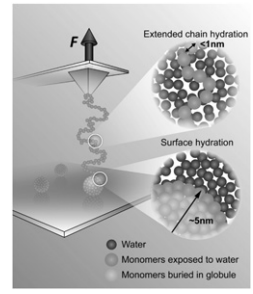
Hydrophobic interaction governs self-assembly in many natural and synthetic molecular systems. A signature of hydrophobicity is the temperature dependence of hydration free energy that varies with solute size. We report the first experimental evaluation of such signature in a single hydrophobic polymer, which tests key assumptions in models of hydrophobic interactions in protein folding. The hydration free energy required to extend three hydrophobic polymers with differently sized aromatic side chains was directly measured by single molecule force spectroscopy. The results showed that the hydration free energy per monomer is strongly dependent on temperature, and the temperature dependence profiles are distinct among the three hydrophobic polymers as a result of a hydrophobic size effect at the subnanometer scale. In addition, we will how surfaces with different hydrophobicity influence the hydration free energy and conformation of hydrophobic polymers adsorbed on it, which serves as a model to study the behaviour of surface adsorbed proteins.



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Scaffold Protein Tethering Ability under Load: FAK's FERM Domain Mechanical Properties V. Binding Site

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Mechanotransductive scaffolding proteins are tethered and thus subjected to mechanical loads that potentially induce partial or total unfolding. Focal adhesion kinase (FAK) is regulated by mechanical stimulation through extracellular matrix (ECM) proteins and actin cytoskeleton contractility. FAK is composed of three major domains: two of which putatively perform tethering (the FERM and FAT domains) while the central kinase domain is catalytically active in a wide variety of cell motility/invasion pathways upon activation. The so-called ''basic patch'' is the ligand-binding site on FERM's F2 subdomain, which is connected, via an unstructured loop, directly to FERM's F3 subdomain and distally to the kinase. As a mechanically competent tether, the FERM domain must carry loads between the basic patch and the F3 subdomain's C-terminal. A key question is whether these subdomains lose their tertiary structure under load_and therefore unwrap into a ''beads on a string'' configuration_and, if so, what consequences this has for ligand-binding subdomain stability. Towards an understanding of the FERM domain's ability to tether a mechanically competent FAK (pdb: 2al6), FERM's unfolding pathways are studied via Steered Molecular Dynamics (SMD). SMD simulations of the unfolding process reveal force peaks, extended conformations of intermediate states, and intramolecular load pathways. Loads are applied to FERM's C-terminal and a set of residues in the basic patch known to bind to both phospholipids and phosphopeptides. By differentially applying loads to the basic patch's secondary structures, unfolding behavior, including both force levels and intermediate states, is revealed. Pulling-mode simulations_mimicking AFM_identify unfolding intermediate states; constant-force-mode simulations probe the structural behavior of identified intermediates. Given the diversity of ligands known to bind to the basic patch, mechanical behavior as a function of binding-site secondary structure is crucial for understanding mechanotransduction.